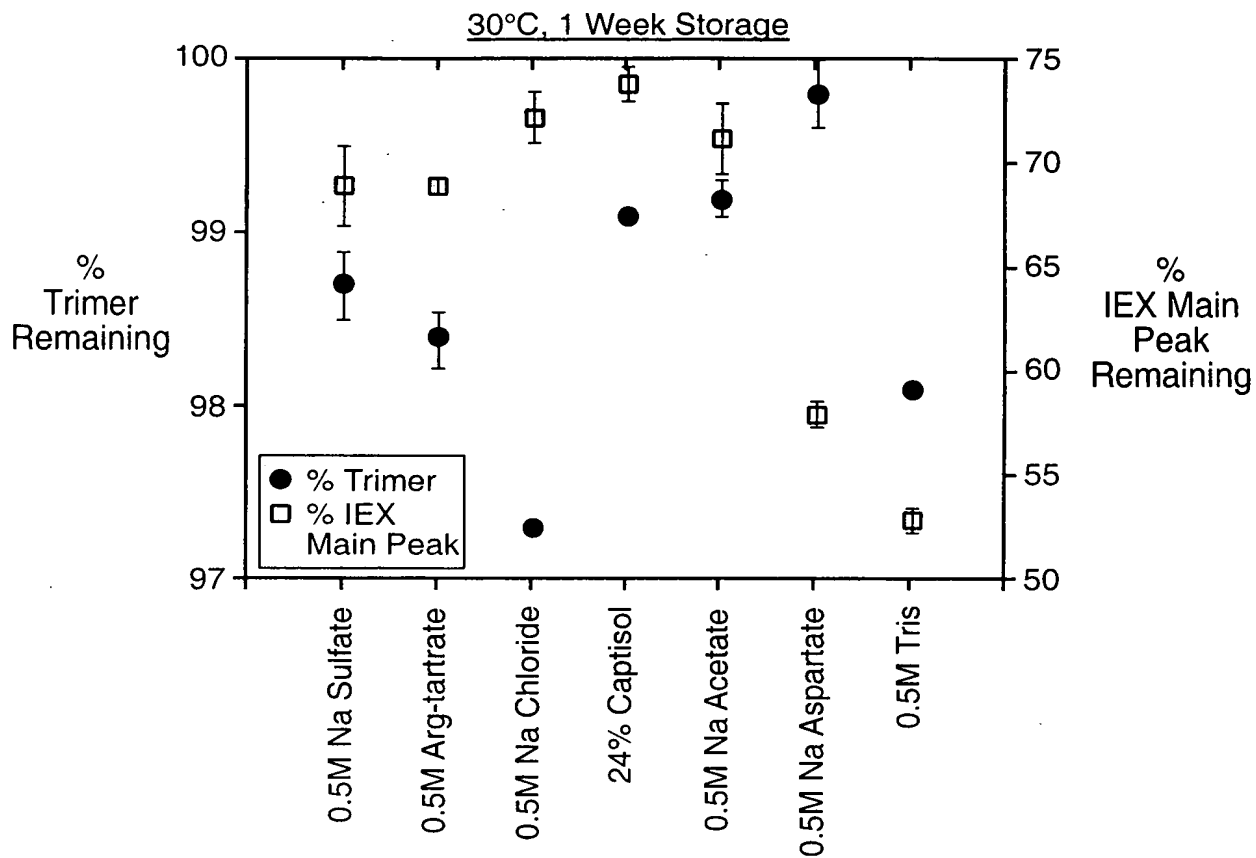


1 TTTCTCTACTGACTATAAAAGAAATAGAGAAGGAAGGGCTTCAGTGACCGGCTGCCTGGCTGACTTACAGCAGTCAGACTCTGACAGGATC  
 1 ATGGCTATGATGGAGGTCCAGGGGGGACCCAGCCTGGGACAGACCTGCGTGTGATCGTGTATCTTCACAGTGTCTCTGACGTCTCTCTGT  
 1 MetAlaMetMetGluValGlnGlyGlyProSerLeuGlyGlnThrCysValLeuIleValIlePheThrValLeuLeuGlnSerLeuCys  
 181 GTGGCTGTAACTTACGTGTACTTTACCAACGAGCTGAAGCAGATGCAGGACAAGTACTCCAAAAGTGGCATTGCTTGTCTTCTTAAAAAGAA  
 31 ValAlaValThrTyrValTyrPheThrAsnGluLeuLysGlnMetGlnAspLysTyrSerLysSerGlyIleAlaCysPylLeuL6sGlu  
 271 GATGACAGTTATTGGGACCCCAATGACGAAGAGAGTATGAACAGCCCTGCTGGCAAGTCAAGTGGCAACTCCGTCAGCTCGTTAGAAAG  
 61 AspAspSerTyrTrpAspProAsnAspGluGluSerMetAsnSerProCysTrpGlnValLysTrpGlnLeuArgGlnLeuValArgLys  
 361 ATGATTTTGAGAACCTCTGAGGAAACCATTTCTACAGTTCAAGAAAAGCAACAAAATATTTCTCCCTAGTGAGAGAAAGAGGTCCNCAG  
 91 MetIleLeuArgThrSerGluGluThrIleSerThrValGlnGluLysGlnAsnIleSerProLeuValArgGluArgGlyProGln  
 451 AGAGTAGCAGCTCACATAACTGGGACCAGAGGAAGAACAACATTTGTCTTCTCCAACTCCAAGAATGAAAAGGCTCTGGGCCGCAAA  
 121 ArgValAlaAlaHisIleThrGlyThrArgGlyArgSerAsnThrLeuSerSerProAsnSerLysAsnGluLysAlaLeuGlyArgLys  
 541 ATAACTCTGGGAATCATCAAGGAGTGGGCATTCTCTGAGCAACTTGCACTTGAGGAATGGTGAACCTCATCCATGAAAAAGGG  
 151 IleAsnSerTrpGluSerSerArgSerGlyHisSerPheLeuSerAsnLeuHisLeuArgAsnGlyGluLeuValIleHisGluLysGly  
 631 TTTTACTACATCTATCCCAAACATACTTTTCGATTTTCAGGAGGAATAAAAGAAAACACAAAGAACGACAAACAATGGTCCAATATATT  
 181 PheTyrTyrIleTyrSerGlnThrTyrPheArgPheGlnGluIleLysGluAsnThrLysAsnAspLysGlnMetValGlnTyrIle  
 721 TACAAAATACACAAAGTTATCCTGACCCCTATATTGTTGATGAAAAGTGCTAGAAATAGTTGTGGTCTAAAGATGCAGAAATATGGACTCTAT  
 211 TyrLysTyrThrSerTyrProAspProIleLeuLeuMetLysSerAlaArgAsnSerCysTrpSerLysAspAlaGluTyrGlyLeuTyr  
 811 TCCATCTATCAAGGGGGAATATTGAGCTTAAGGAAAATGACAGAAATTTTGTCTGTAAACAAATGAGCACTTGATAGACATGGACCAT  
 241 SerIleTyrGlnGlyIlePheGluLeuLysGluAsnAspArgIlePheValSerValThrAsnGluHisLeuIleAspMetAspHis  
 901 GAAGCCAGTTTTCGGGGCCTTTTGTAGTTGGCTAACTGACCTGGAAAAGAAAAGCAATAACCTCAAAGTGACTATTTCAGTTTTCAGGAT  
 271 GluAlaSerPhePheGlyAlaPheLeuValGlyStp  
 991 GATACACTATGAAGATGTTTCAAAAAATCTGACCAAAAAACAAACAGAAA

FIG..1

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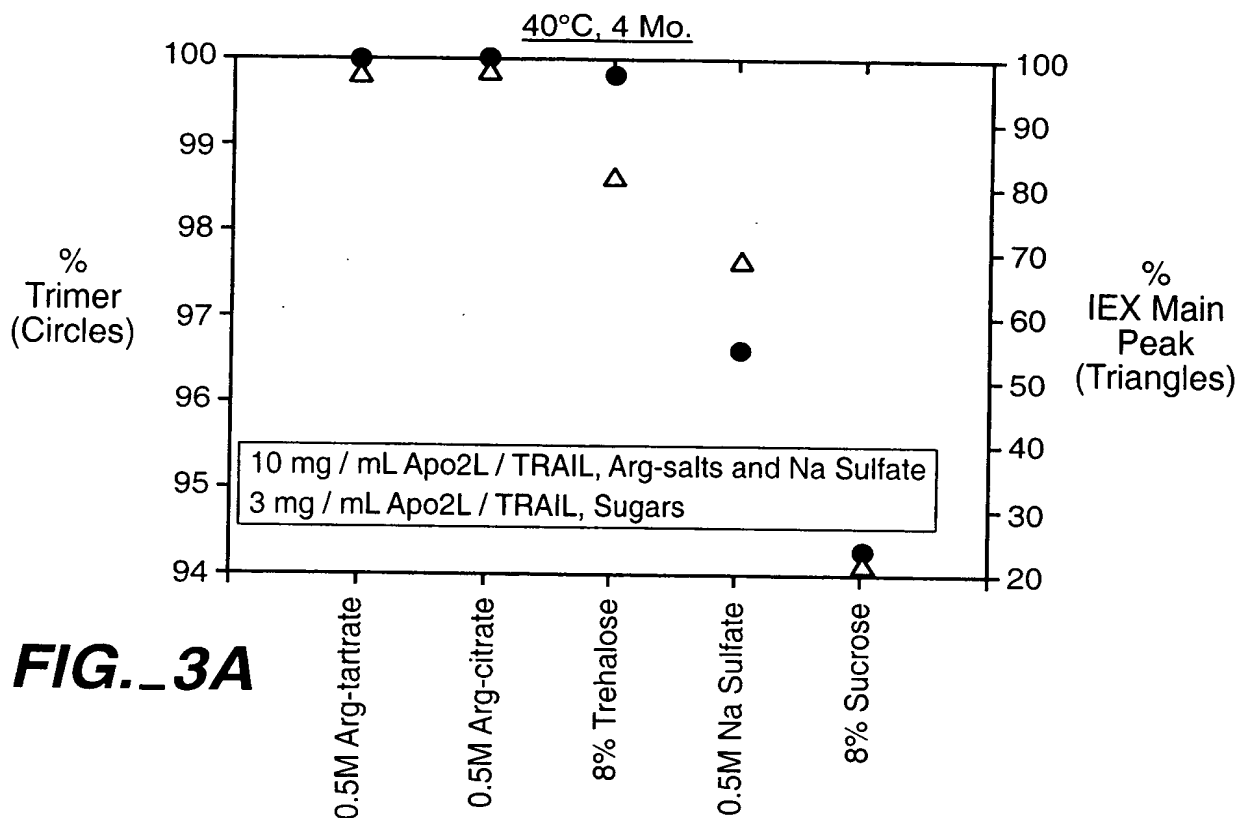
Liquid Stability of Apo2L / TRAIL in Various Preparations Following 1 Week Storage at 30°C.



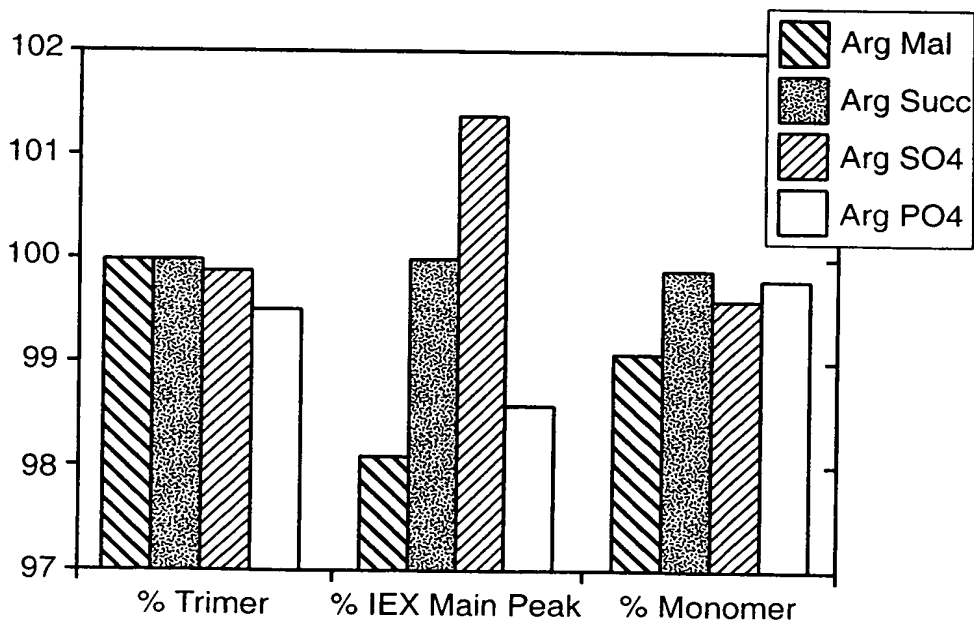
**FIG. 2**



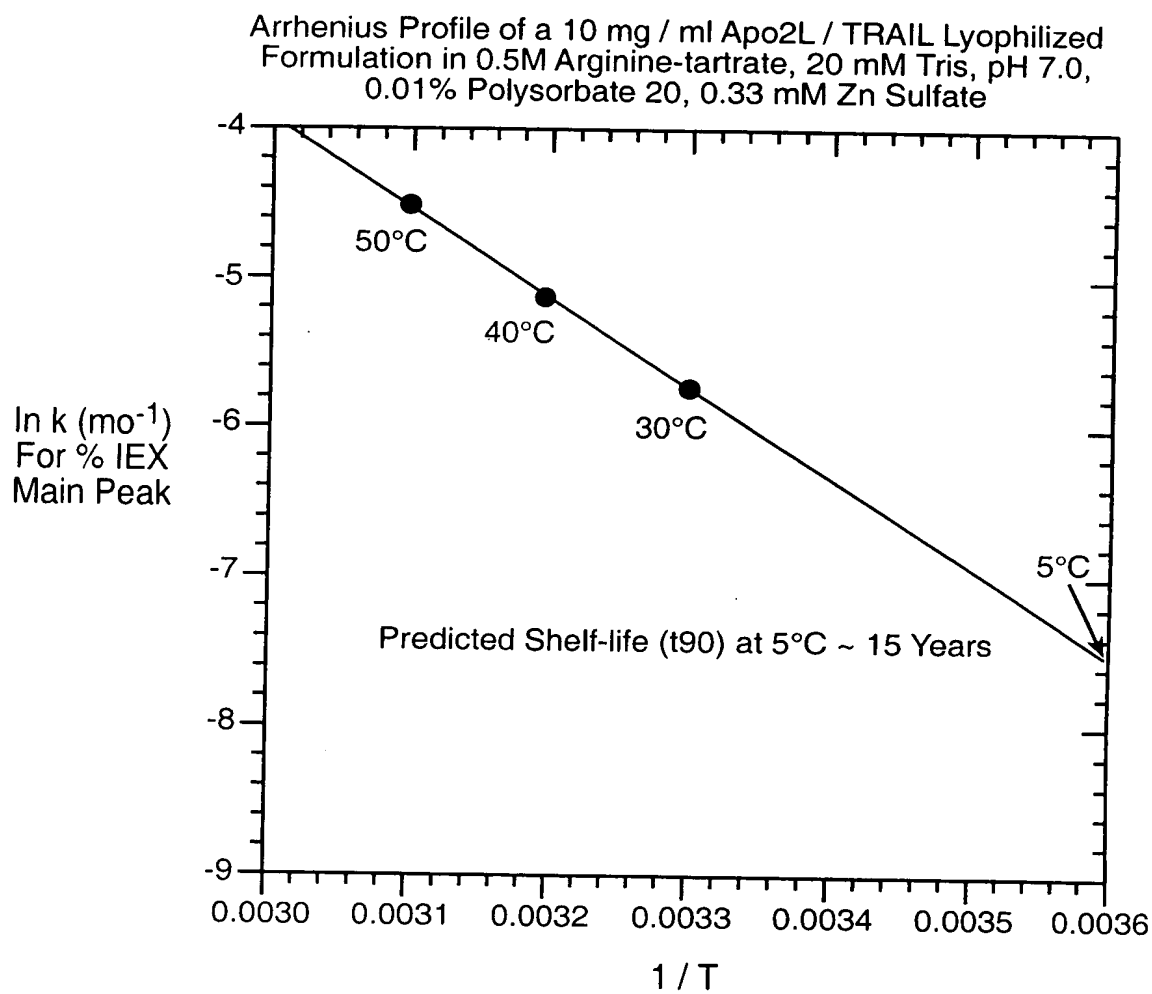
Stability of Lyophilized Apo2L / TRAIL  
Preparations After 4 Months Storage at 40°C.



Stability of Various Arginine-salt Containing Lyophilized  
Apo2L / TRAIL Formulations After 1 Month Storage at 50°C

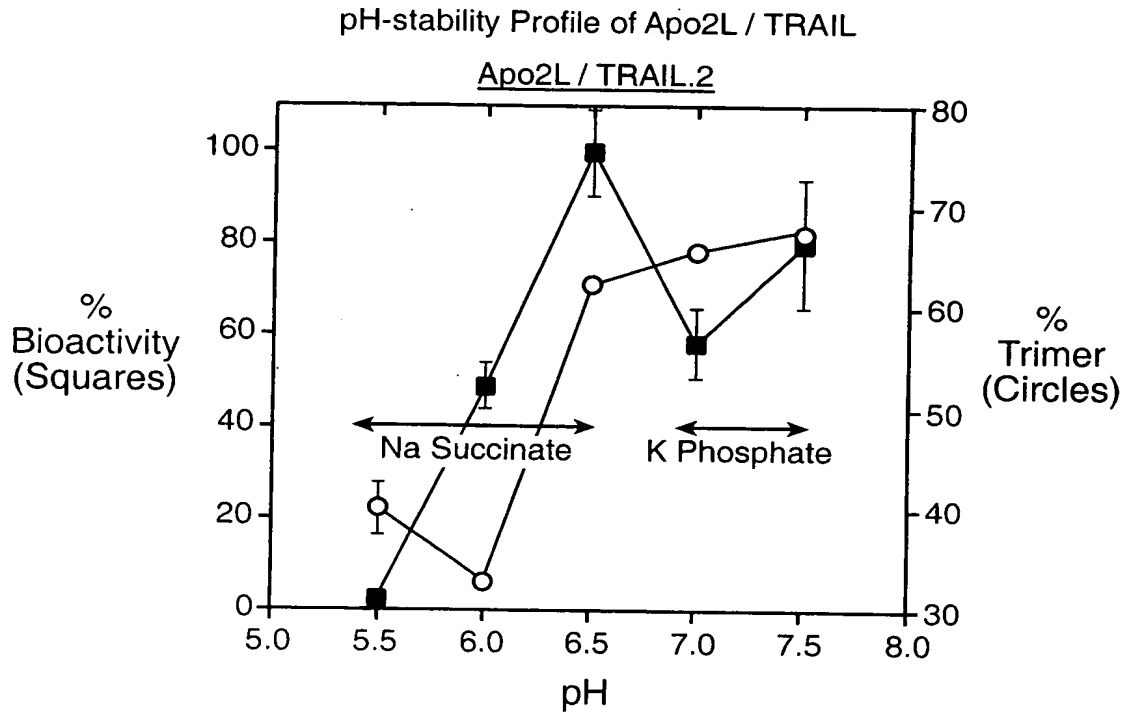
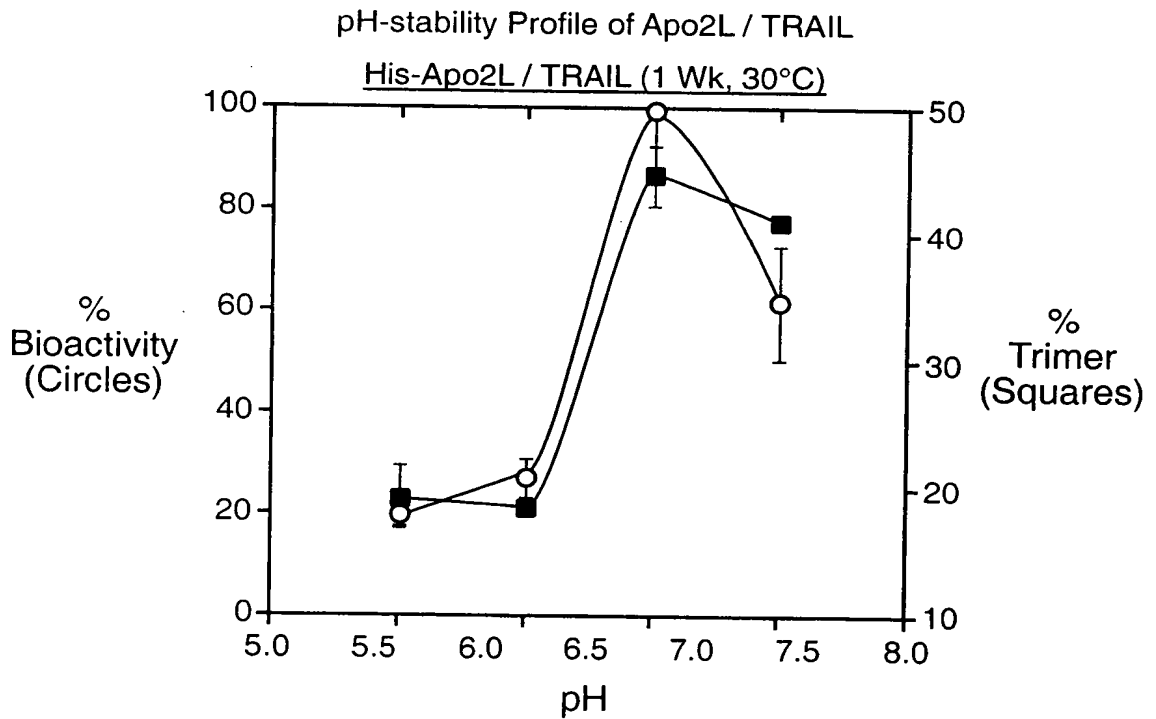


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**FIG.\_3C**

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**FIG.\_4A****FIG.\_4B**

+

Zn Coordination to Apo2L / Trail and Effect of pH

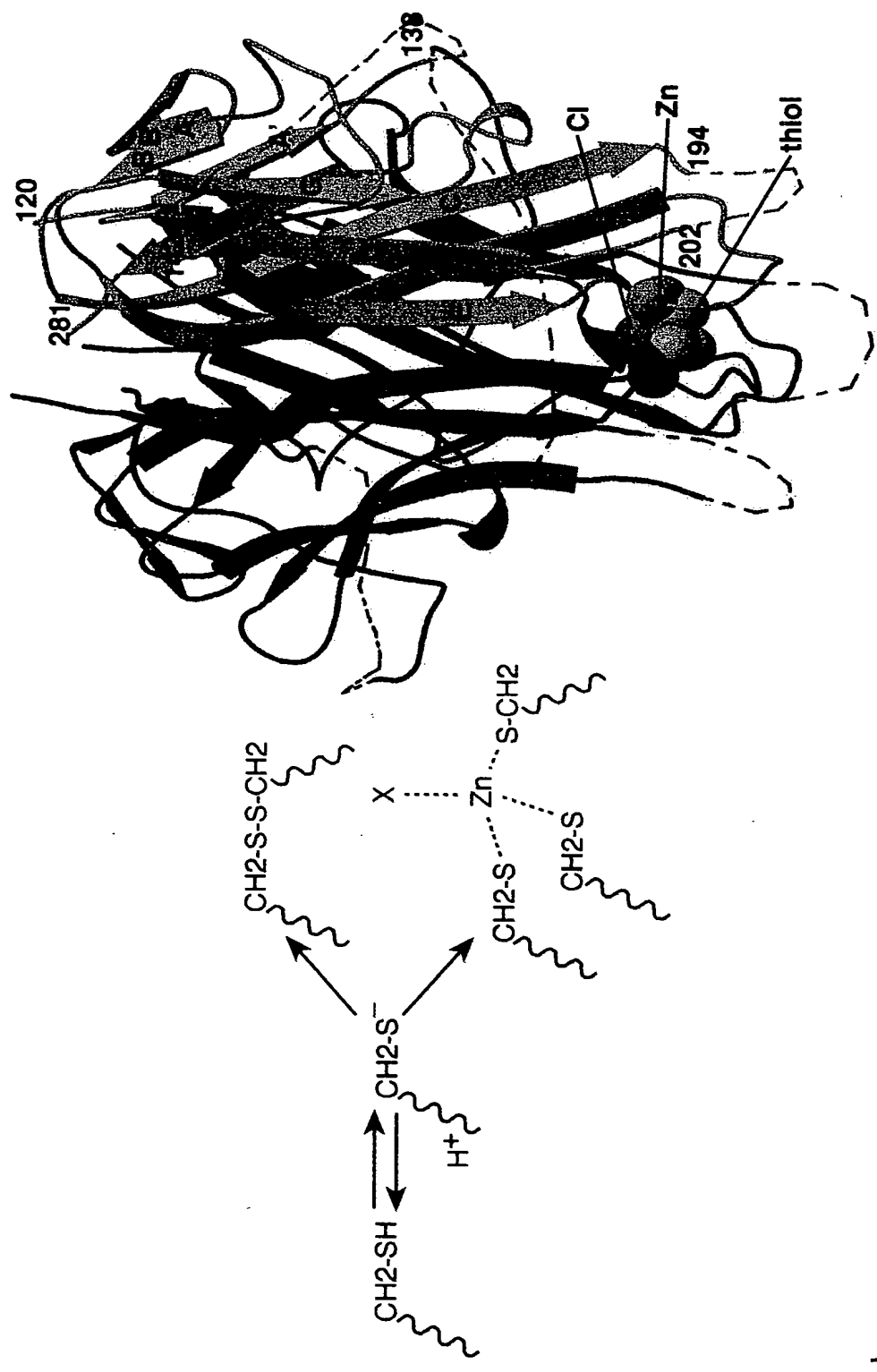
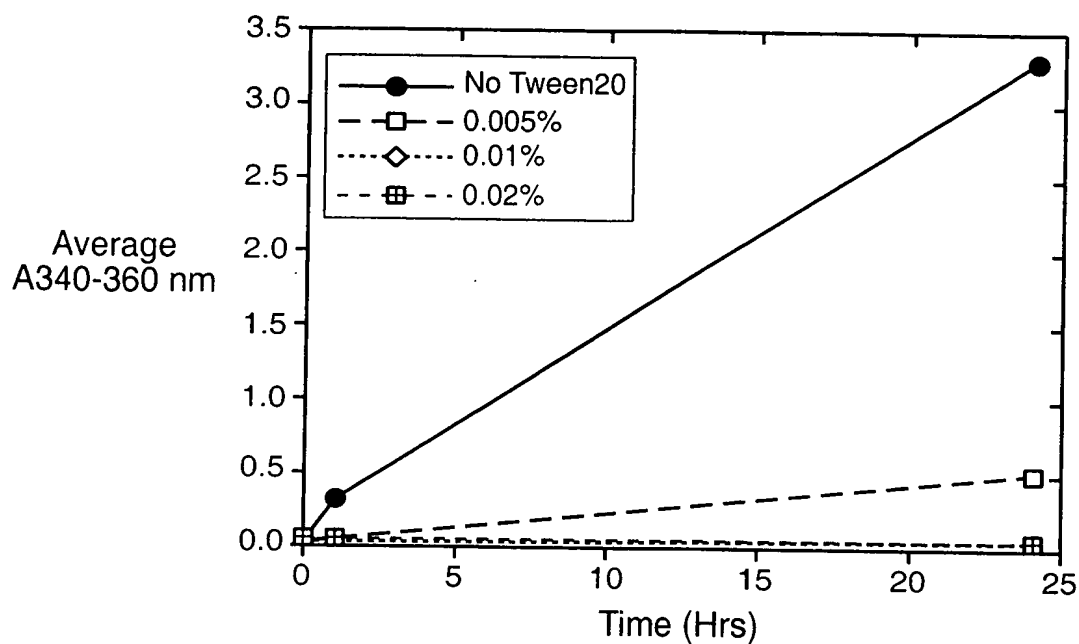


FIG.\_5

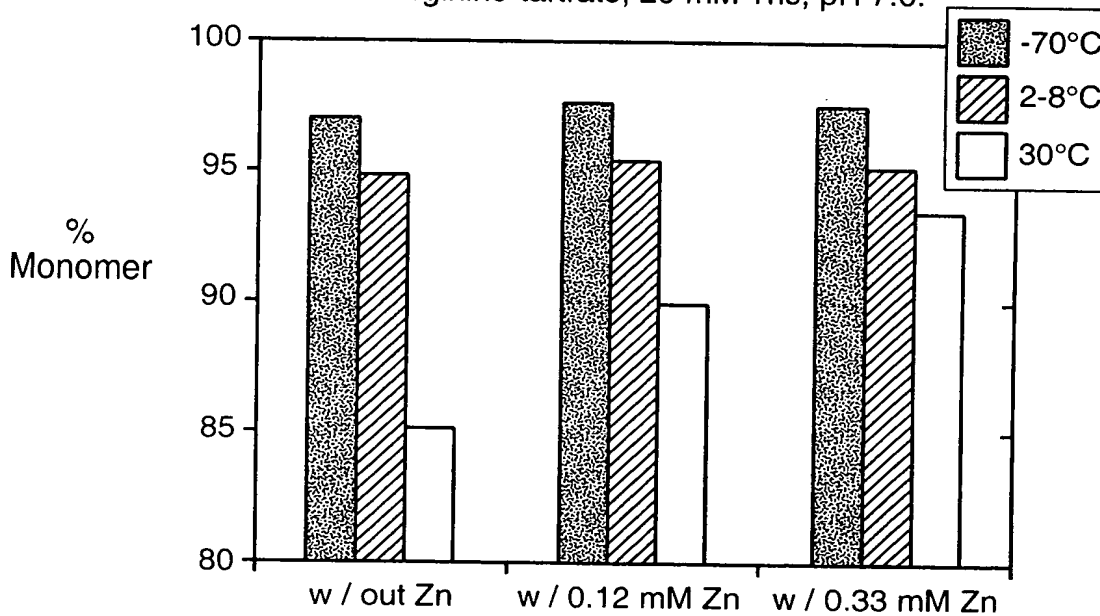


Effect of Polysorbate (Tween) 20 on Stabilization of Apo2L / TRAIL



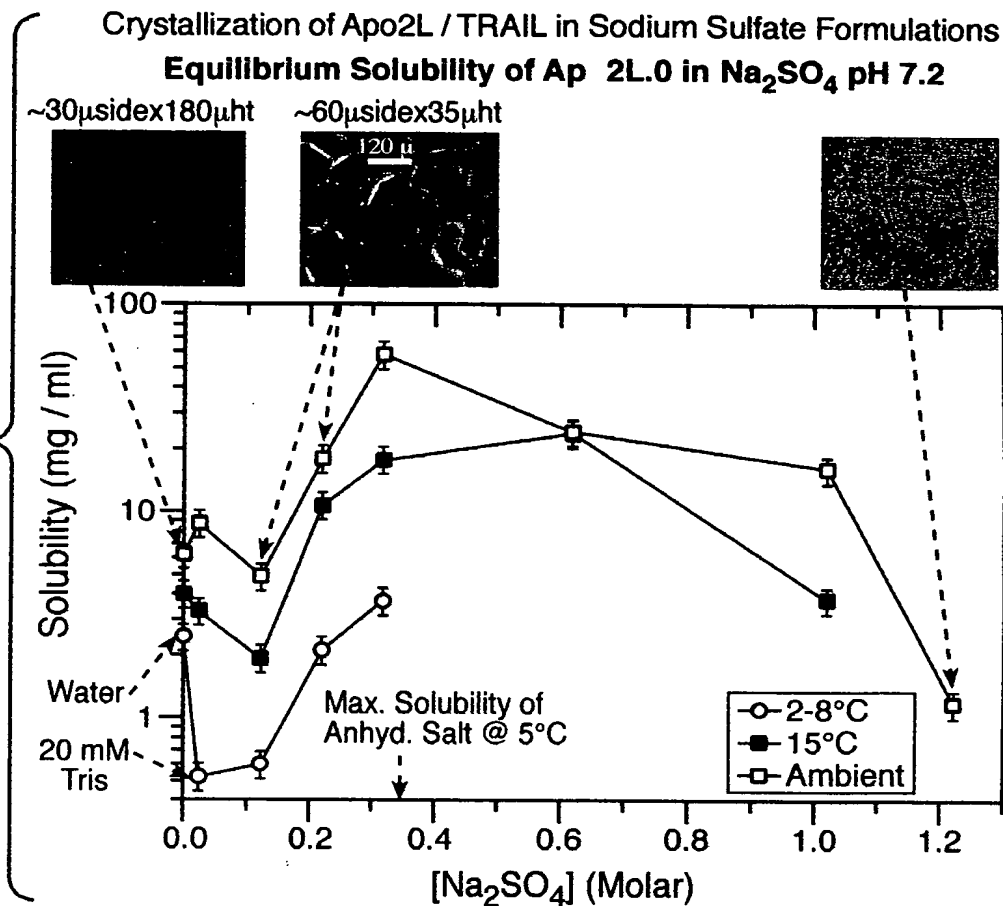
**FIG.\_6**

Effect of Zn on Thermal Stabilization of Apo2L / TRAIL After 2 Months Storage as a Liquid Formulation Containing 0.5M Arginine-tartrate, 20 mM Tris, pH 7.0.

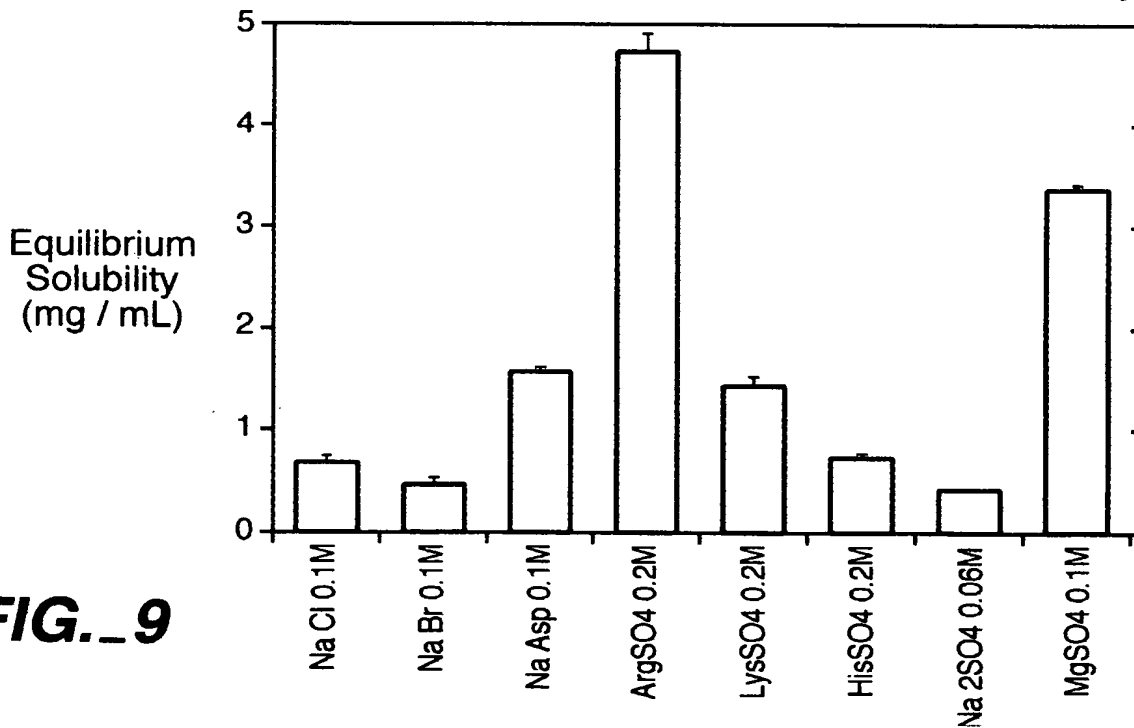


**FIG.\_7**

**FIG.\_8**



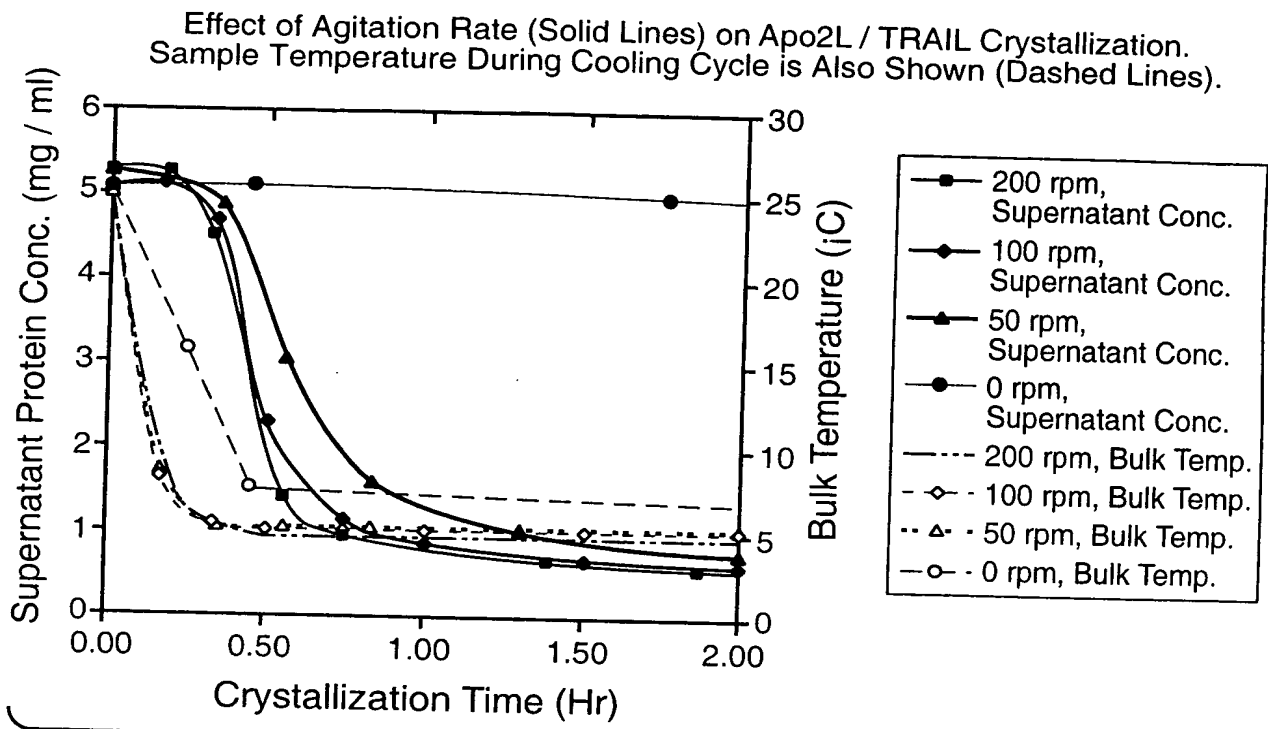
Ion Dependence Crystallization of Apo2L / TRAIL. Crystallization Was Observed in All Salts, but Arginine and Mg Salts Maximized the Protein Solubility in the Narrow Range of 10-11.5 mS / cm Conductivity.



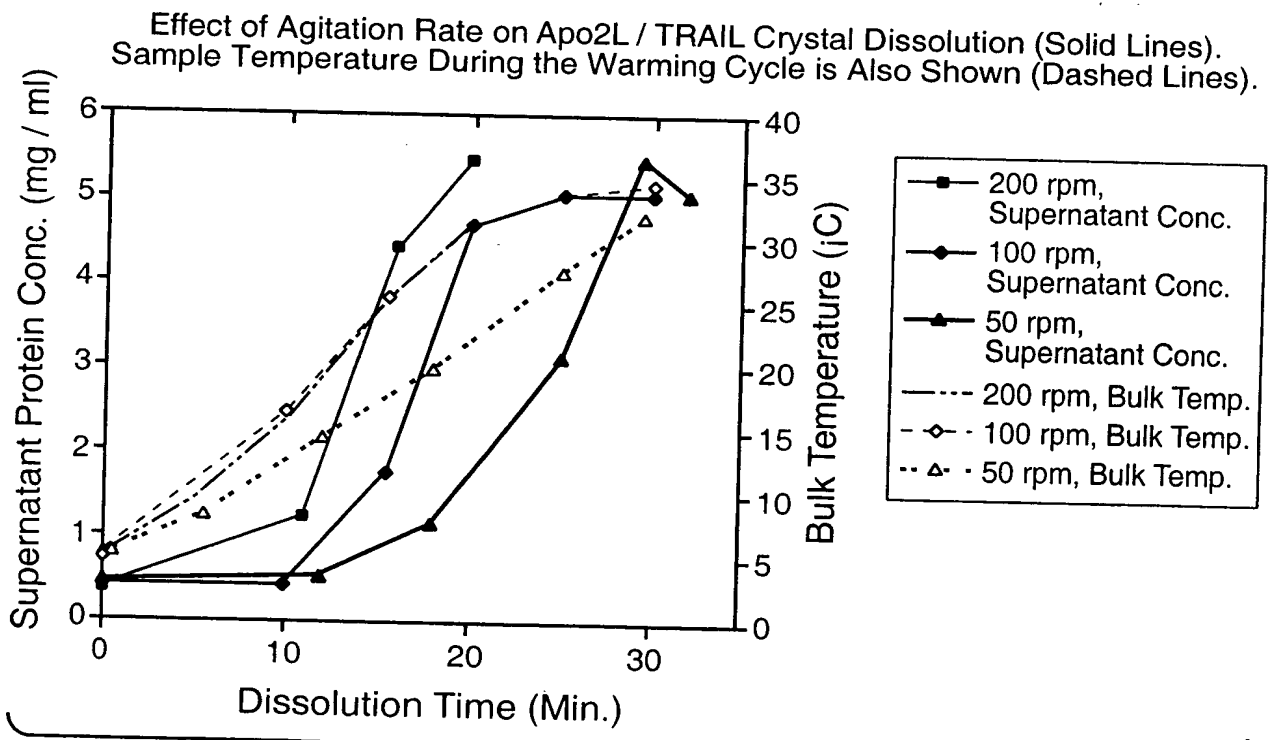
**FIG.\_9**



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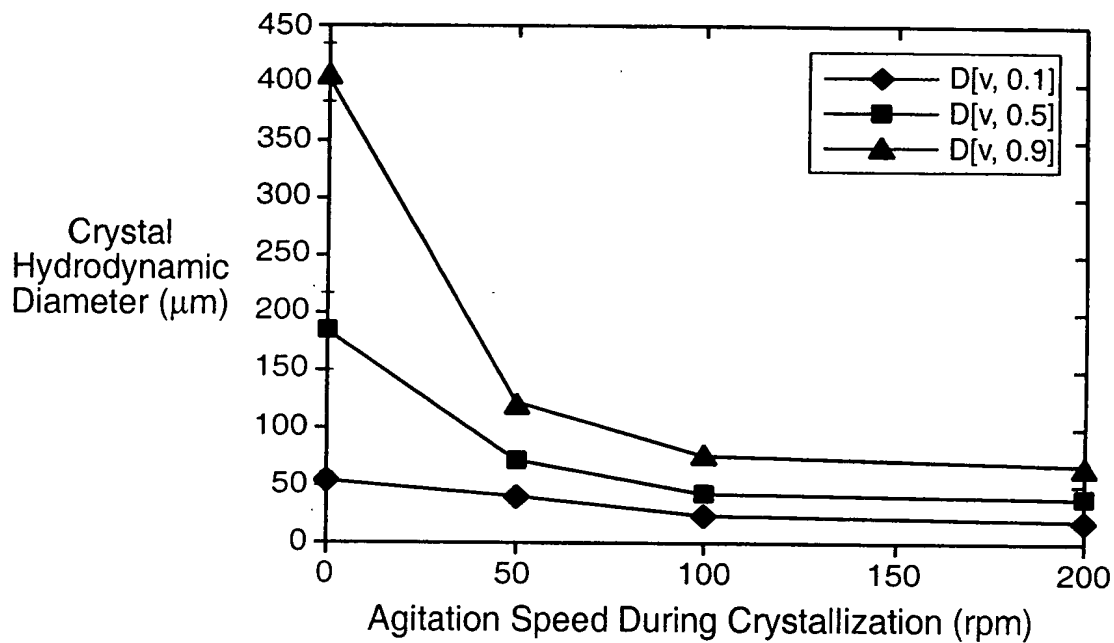
**FIG. 10A**



**FIG. 10B**



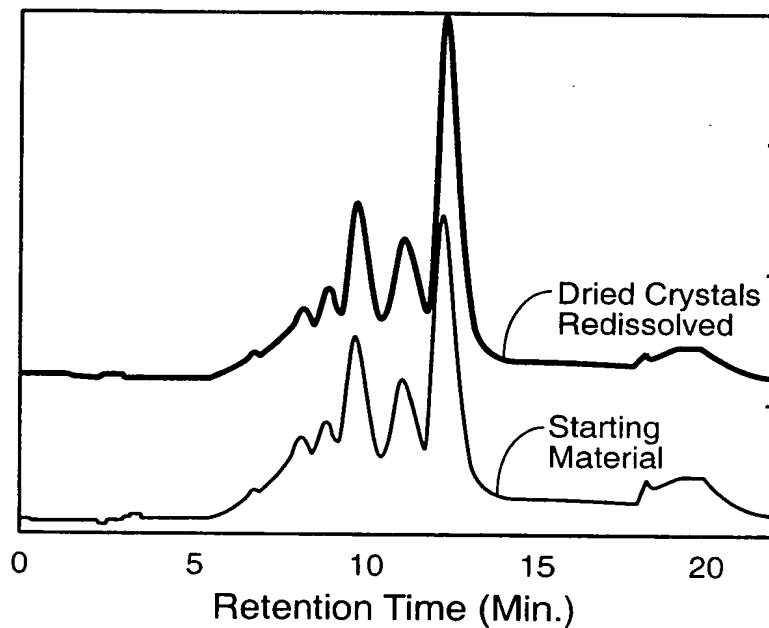
Effect of Agitation Rate on Apo2L / TRAIL Crystal Size Distribution



**FIG.\_10C**

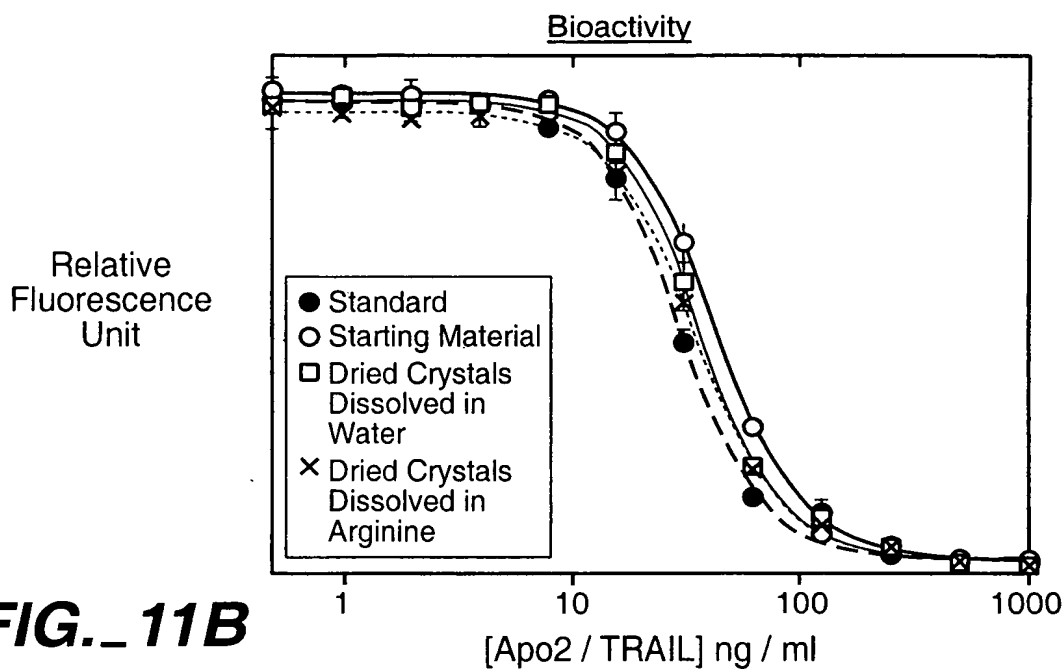
IEX Profile of Apo2L / TRAIL after Reconstitution of Vacuum Dried Crystals

IEX Profile

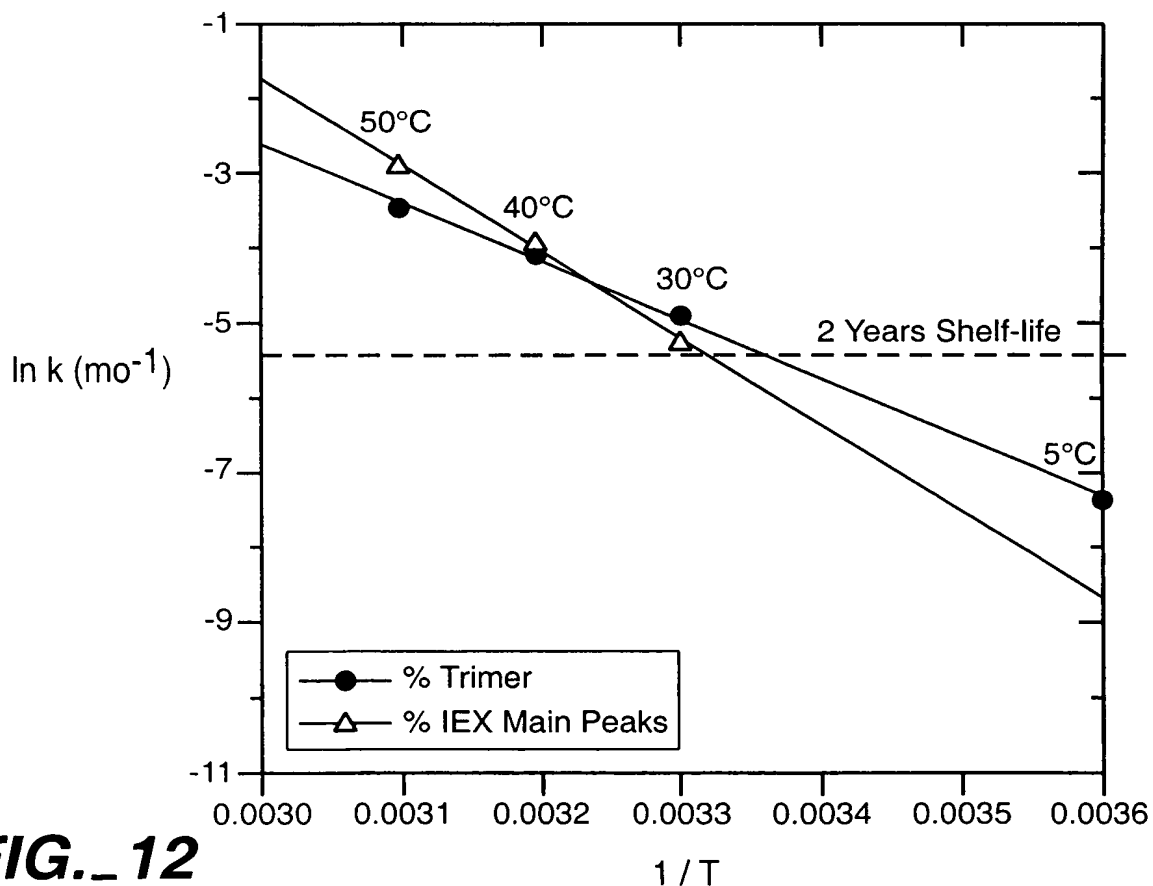


**FIG.\_11A**

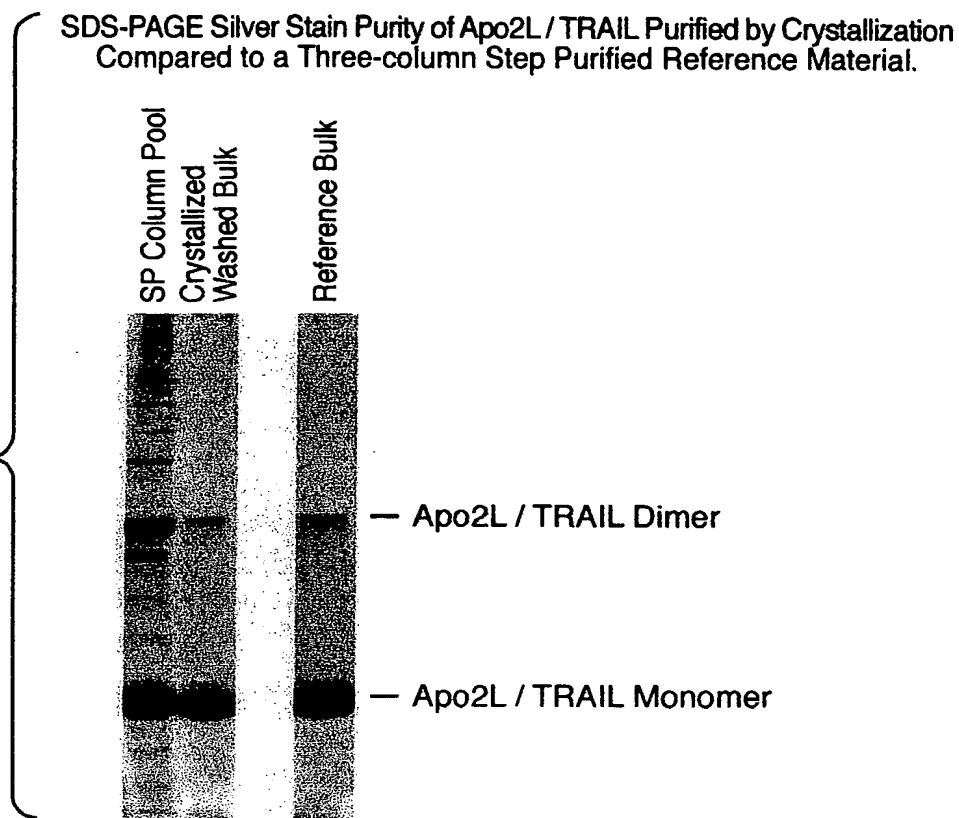
# Bioactivity of Apo2L / TRAIL after Reconstitution of Vacuum Dried Crystals.



## Arrhenius Profile of a 20 mg / ml Apo2L / TRAIL Lyophilized Formulation in 0.2M Na Sulfate, 20 mM Tris, pH 7.2, 0.01 % tween 20.



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**FIG. 13**

Effect of Salt Type on Crystallization of Partially Purified Apo2L / TRAIL. After Partial Purification of E. Coli Clarified Lysates on Sp-sepharose Cation Exchange Column, the Protein Was Eluted At 5-10 mg / ml in 20 mM Tris, pH 8 and 0.2M of One of the Salts Shown. The Samples Were Stored At 2-8°C For 3-7 Days. An Aliquot Was Then Filtered and the Soluble Protein Concentration was Measured by UV Spec Scan.

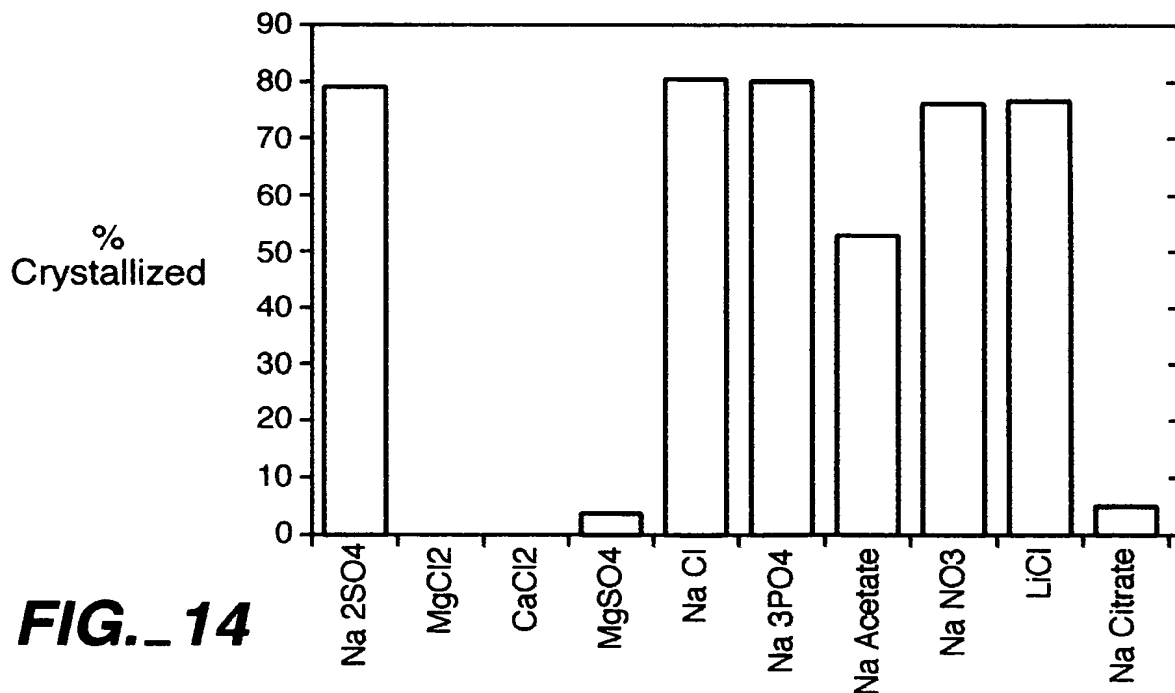
**FIG. 14**

Figure 15A

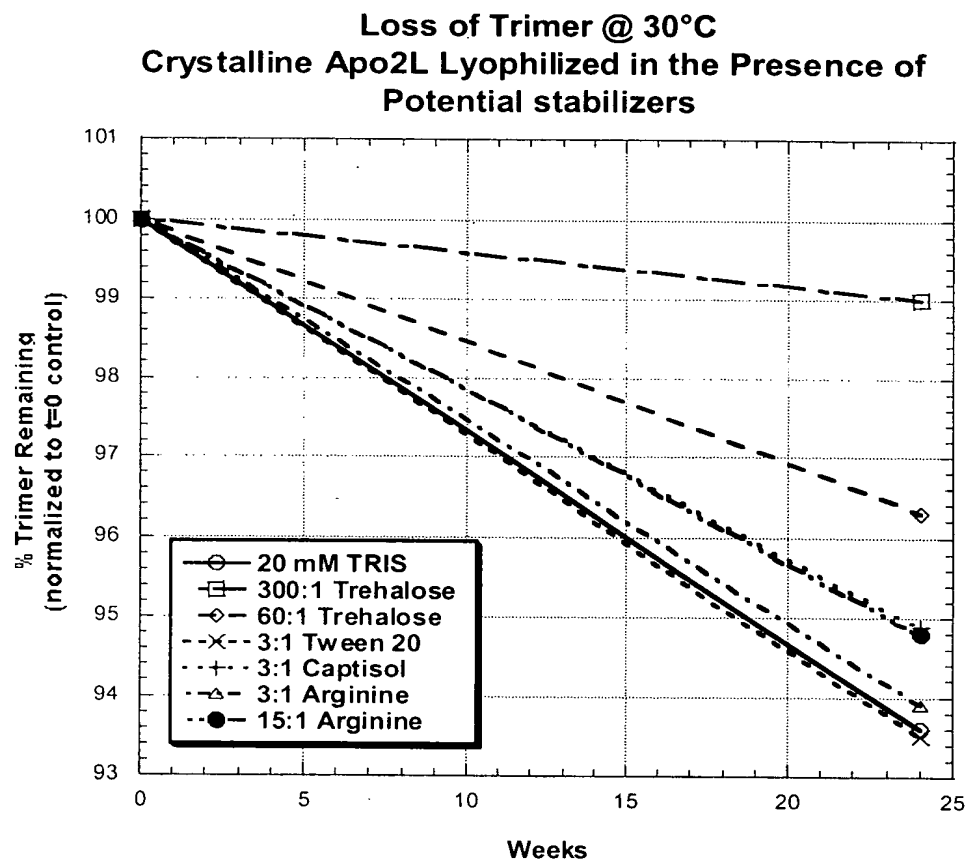


Figure 15B

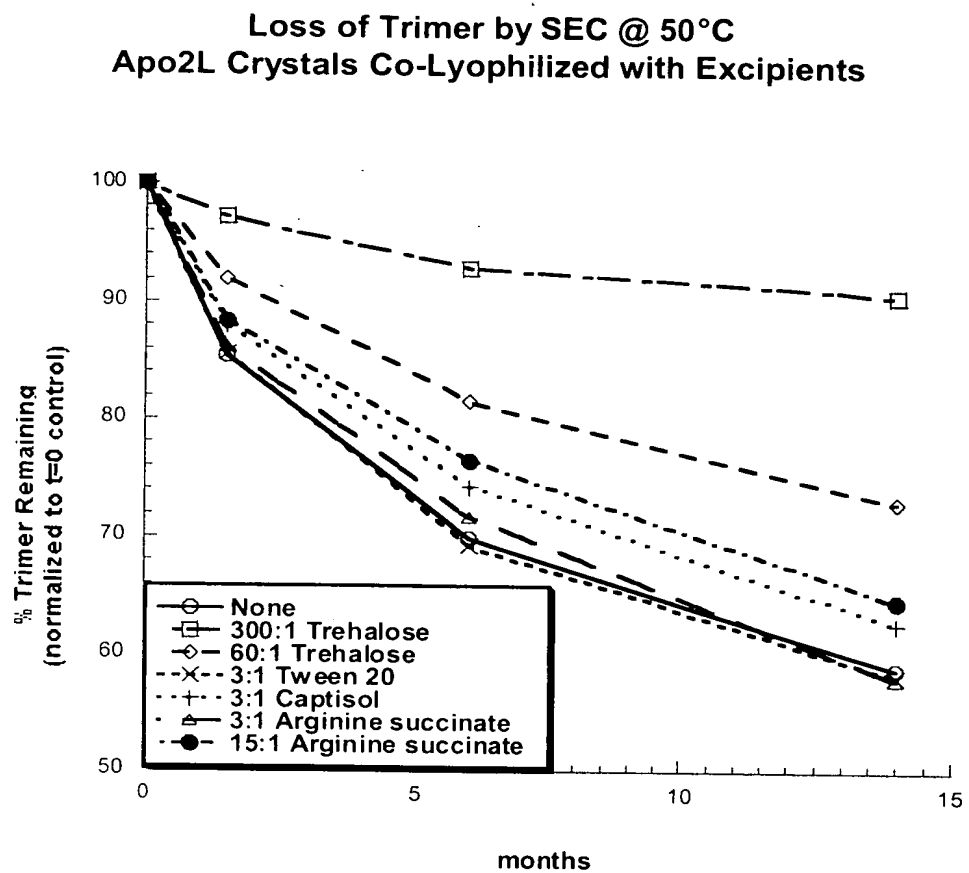


Figure 15C

**Increase in % Non-Reducible Dimer @ 50°C**  
**Crystalline Apo2L Lyophilized in the Presence of**  
**Potential Stabilizers**

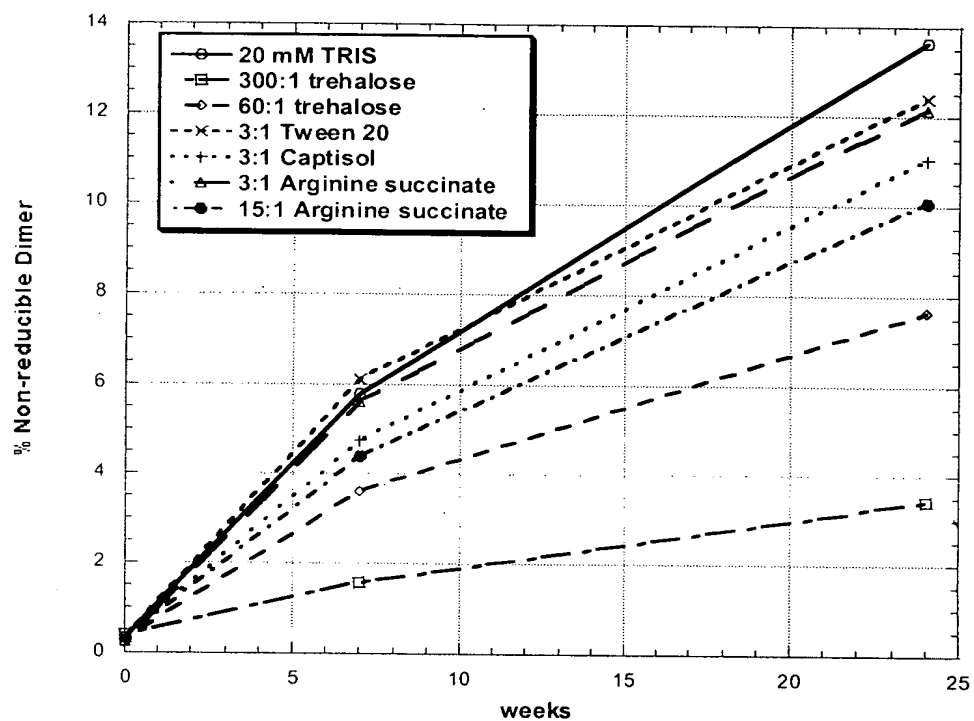


Figure 16A

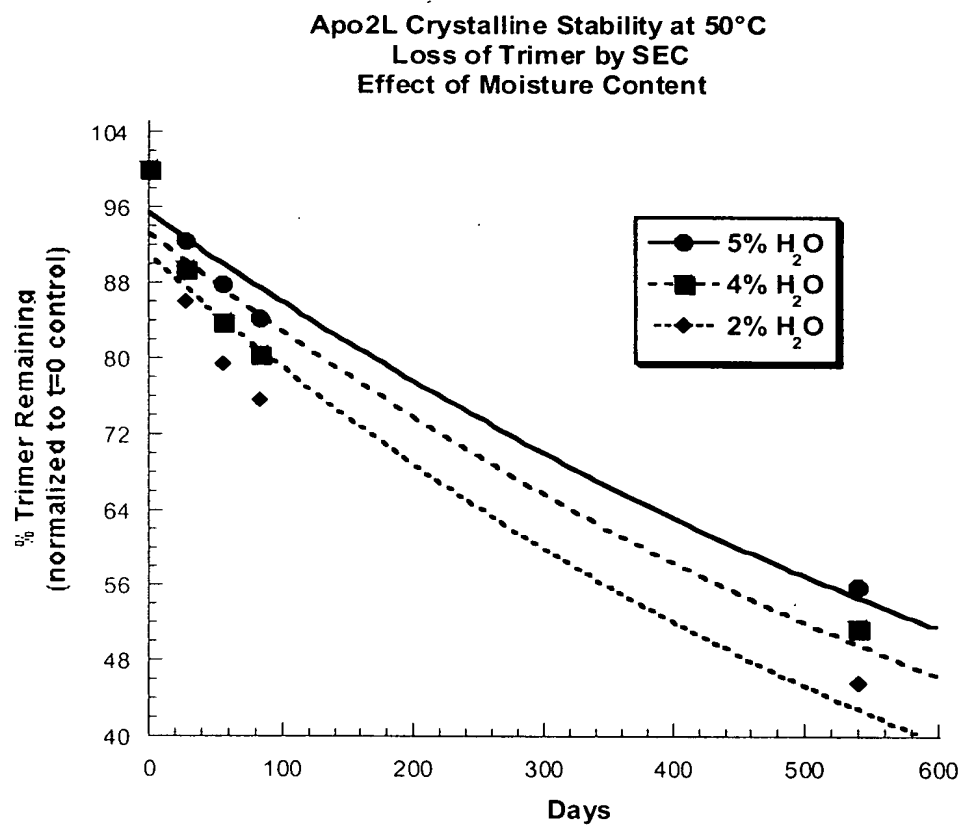




Figure 16B.

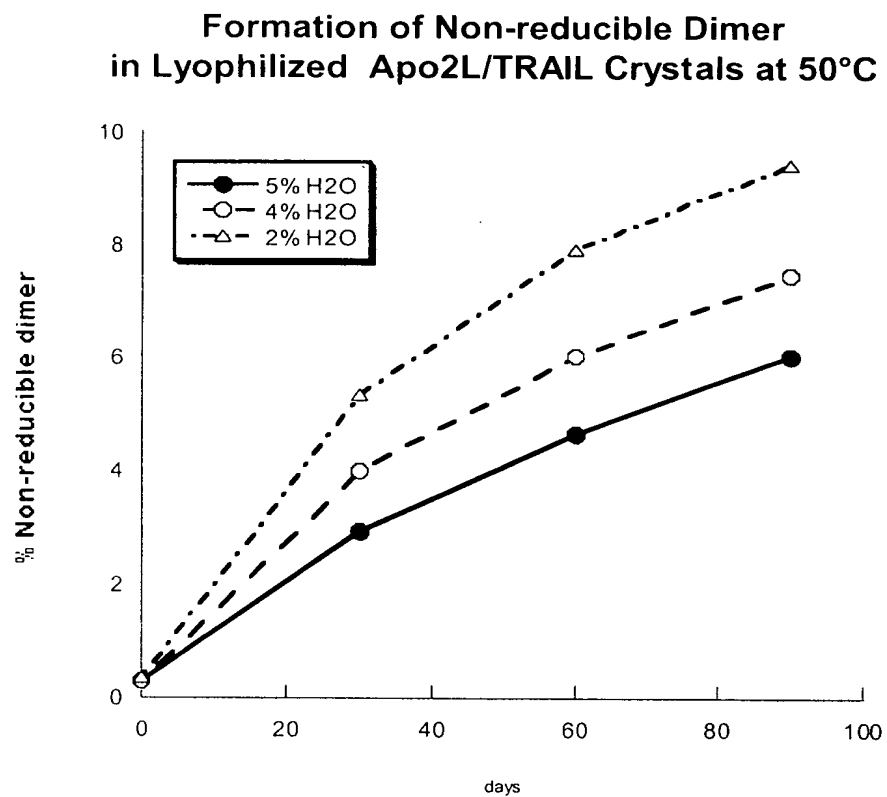


Figure 17A

**Lyophilized Apo2L/TRAIL Crystals  
SDS-SEC Chromatograms  
2.5% Residual Moisture**

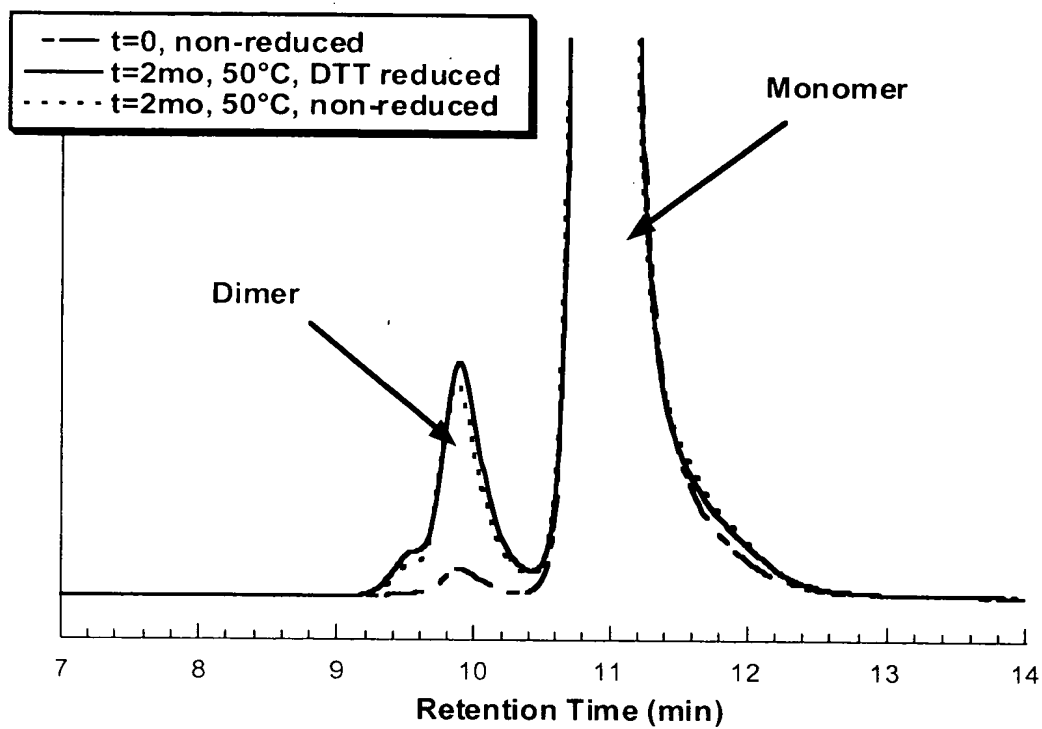


Figure 17B

**Lyophilized Apo2L/TRAIL Crystals  
SDS-SEC Chromatograms  
12% Residual Moisture**

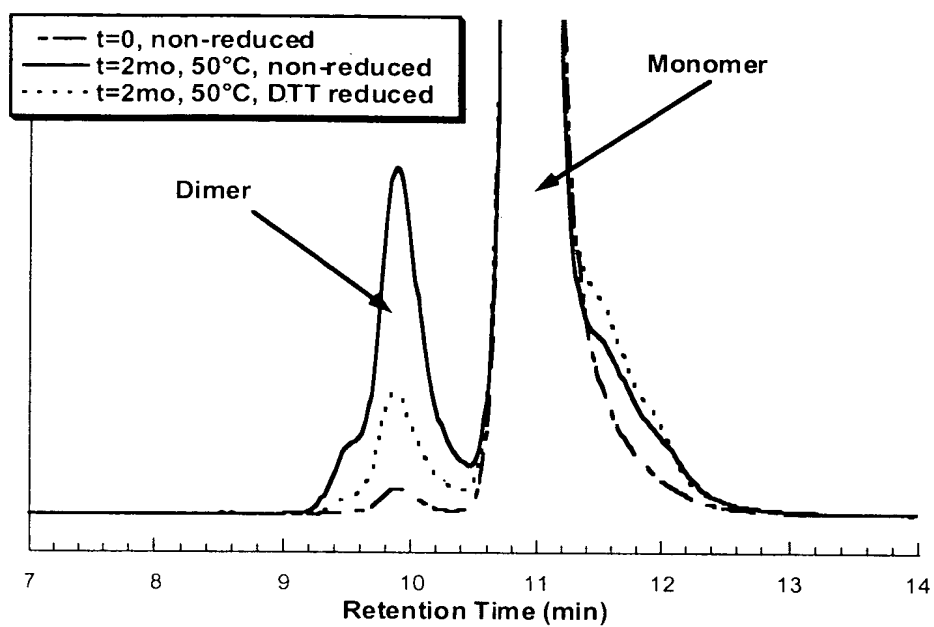


Figure 18A

**SDS-SEC Chromatograms of Hexamer Fraction  
Collected From Apo2L Crystals Containing 2.5% H<sub>2</sub>O**

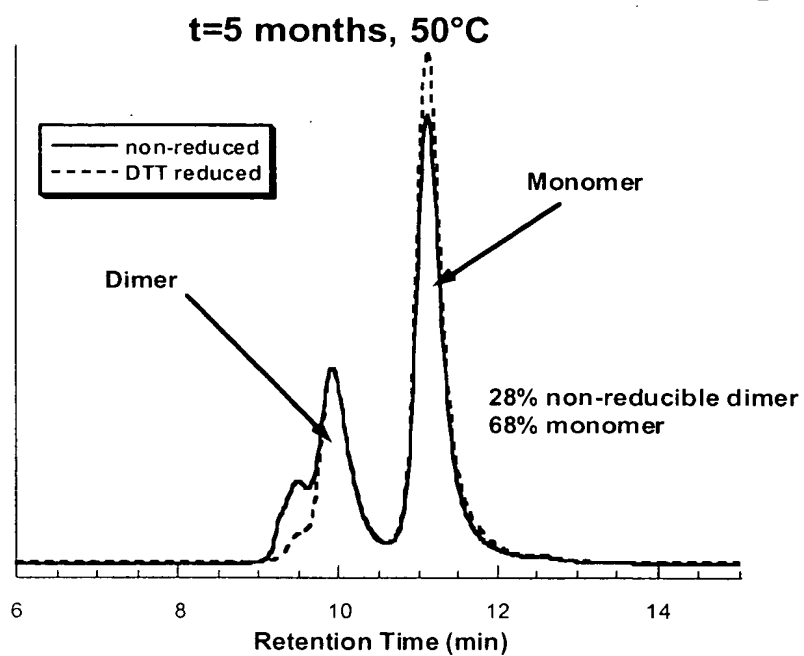


Figure 18B

**SDS-SEC Chromatograms of Hexamer Fraction  
Collected From Apo2L Crystals Containing 12%  $\text{H}_2\text{O}_2$**

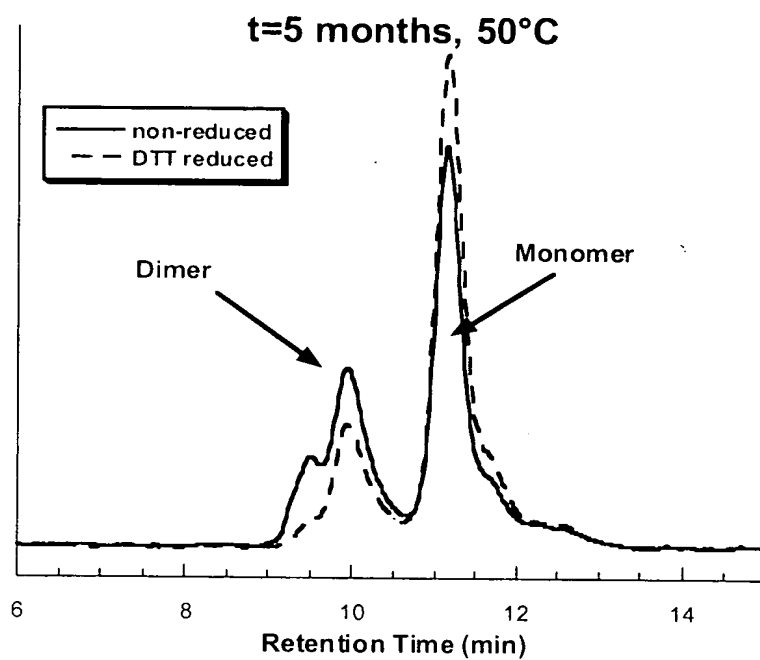


Figure 19A

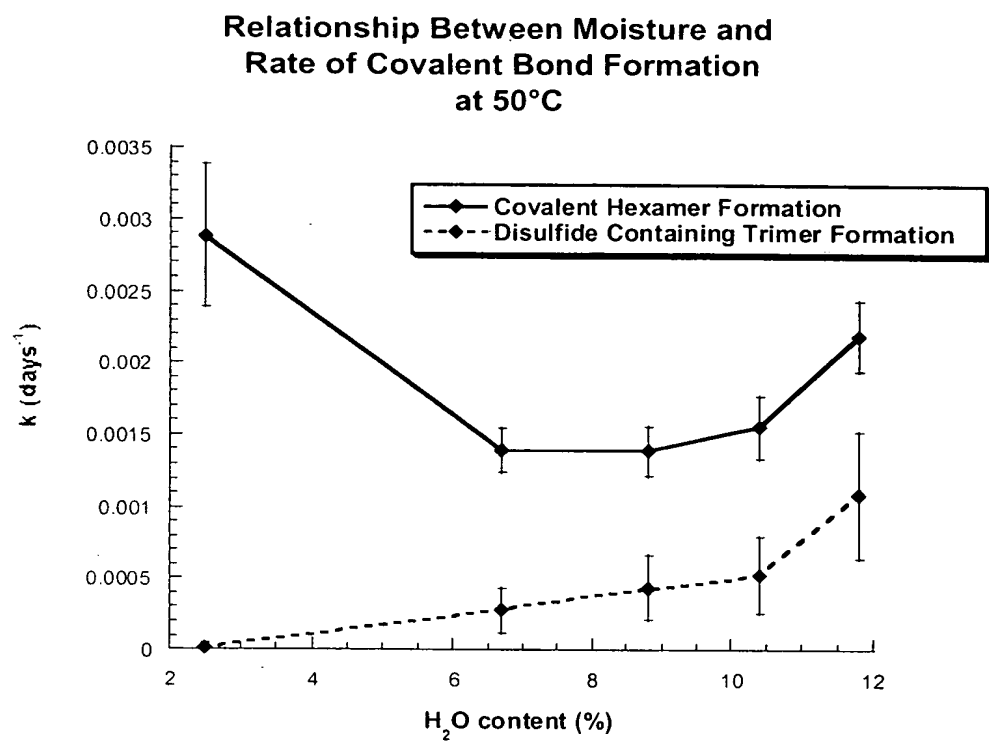


Figure 19B

